Dissertation

On

“STUDY TO DESCRIBE THE ETIOLOGICAL CAUSES OF LYMPHADENOPATHY IN PATIENTS ATTENDING TERTIARY CARE CENTRE AMONG SOUTH INDIAN POPULATION”

M.S. BRANCH - I

GENERAL SURGERY

MADRAS MEDICAL COLLEGE
THE TAMILNADU DR MGR MEDICAL UNIVERSITY
CHENNAI

APRIL 2016

CERTIFICATE

This is to certify that the dissertation entitled "**STUDY TO DESCRIBE THE ETIOLOGICAL CAUSES OF LYMPHADENOPATHY IN ATTENDING TERTIARY CARE CENTRE AMONG SOUTH INDIAN POPULATION"** is the bonafide work done by **Dr. G. VARAGUNA PANDIAN** during his MS (General Surgery) course 2013-2016, done under my supervision and is submitted in partial fulfillment for the requirement of the M.S.(BRANCH-I)- General Surgery, April 2016 examination of The Tamilnadu Dr.MGR Medical University

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DECLARATION

I, declare that this dissertation title **"STUDY TO DESCRIBE THE ETIOLOGICAL CAUSES OF LYMPHADENOPATHY IN PATIENTS ATTENDING TERTIARY CARE CENTRE AMONG SOUTH INDIAN POPULATION"** represents a genuine work of mine. The contributions of any supervisors to the research are consistent with normal supervisory practice, and are acknowledged.

I also affirm that this bonafide work or part of this work was not submitted by me or any others for any award, degree or diploma to any other University board, either in India or abroad. This is submitted to The TamilNadu Dr. M.G.R Medical University, Chennai in partial fulfillment of the rules and regulations for the award of Master of Surgery Degree Branch I (General Surgery).

Date:

Place: **Dr. G.VARAGUNAPANDIAN**

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
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CERTIFICATE OF APPROVAL

To
Dr.Varagunapandian G
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Dear Dr.Varagunapandian G

The Institutional Ethics Committee has considered your request and approved your study titled **"Study to describe the etiological causes of lymphadenopathy in patients attending tertiary care centre among South Indian Population" No.11082015.**

The following members of Ethics Committee were present in the meeting held on 04.08.2015 conducted at Madras Medical College, Chennai-3.

1. Prof.C.Rajendran, M.D.,	: Chairperson
2. Prof.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.Sudha Seshayyan, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
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8. Tmt. J.Rajalakshmi, J.A.O. MMC, Ch-3	: Lay Person
9. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10.Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

14/8/15

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
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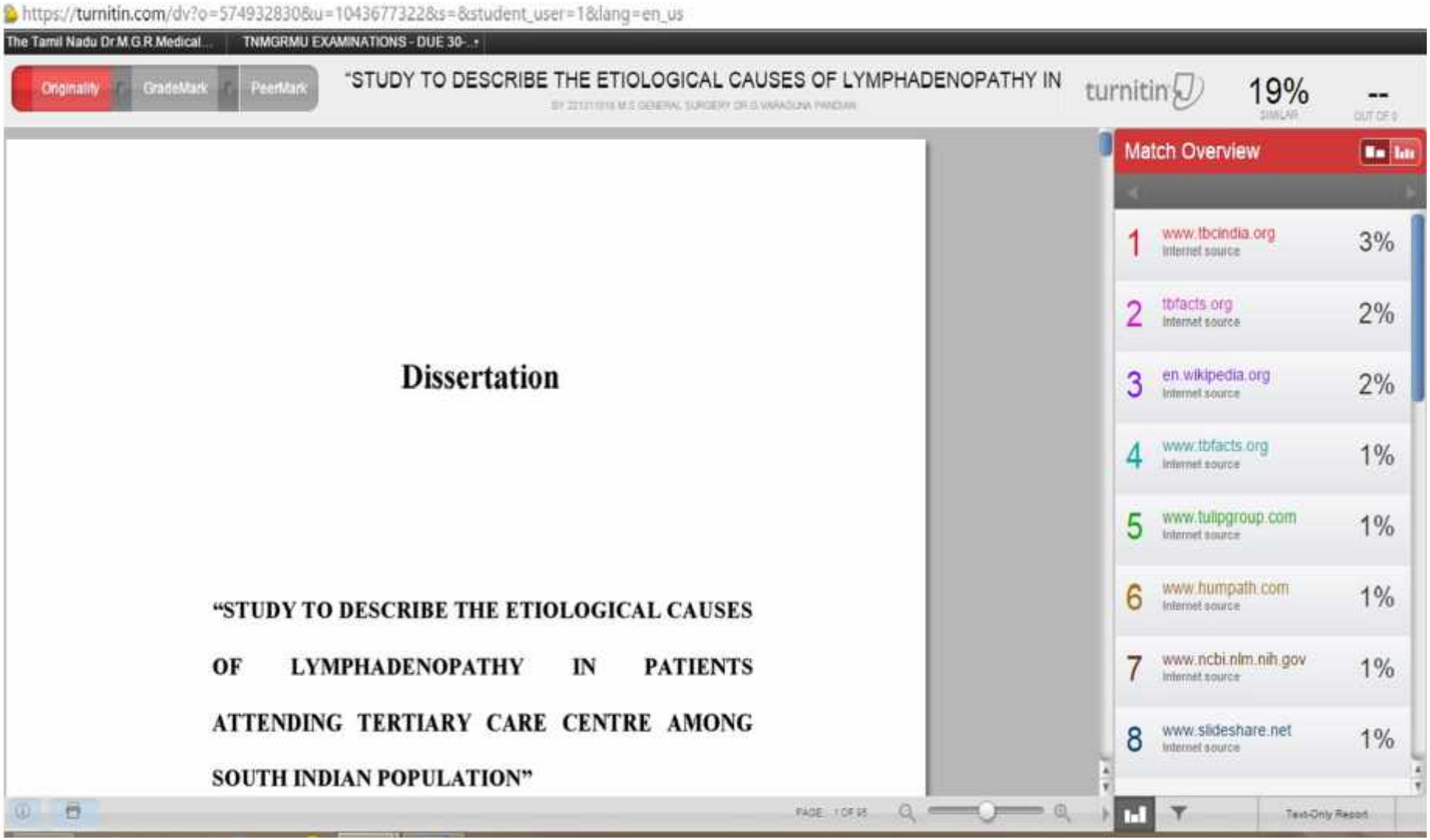
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ACKNOWLEDGEMENT

As I walk down the memory lane, I realize with a deep sense of humility that what I have done now would not have been possible, but for certain luminaries, who have enlightened my path to wisdom.

"Surgery is learnt by apprenticeship and not from textbooks, not even from one profusely illustrated" – Ian Aird.

While I put these words together it is my special privilege and great pleasure to record my deep sense of gratitude to my revered Professor and Guide **Prof. A. Affee Asma. M.S.,** but for whose constant guidance, help and encouragement this research work would not have been made possible. The unflinching academic, moral and psychological support will remain ever fresh in my memory for years to come. Words cannot simply express my gratitude to them for imparting to me the surgical skills that I have acquired.

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My special thanks to my Mentor **Dr. K. Raja, M.D,** Nodal officer, ART center, GHTM, Tambaram, Chennai – 47 for helping me to finish this study.

All along the way, I have been supported and encouraged by all my associate professors and assistant professors who helped me to reach where I am.

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I thank the Dean, MMC & RGGGH for permitting me to conduct this study.

I would be failing in my duty if I do not show my deep sense of gratitude to all the patients who had helped me to become a surgeon and especially those who consented to be part of this study.

With deep reverence, I salute my parents and I thank the Almighty for blessing me with a wonderful family to whom I have dedicated this thesis and leave unsaid what they mean to me.

ABSTRACT

INTRODUCTION:

Lymphadenopathy is a common clinical finding in the Out Patient Department and may be the sign for various etiology of disease pathology. It is important to take proper history taking and examination for diagnosing the etiology of the lymphadenopathy. The etiology varies in different areas. Tuberculosis is the commonest etiology for cervical lymphadenopathy in India and African countries. Most of the studies show common benign etiologies as Non specific reactive lymphadenopathy

METHODS AND METHODOLOGY:

The retrospective and prospective study to analyse study to describe the etiological causes of lymphadenopathy in patients attending tertiary care centre among south Indian population.

RESULTS:

Of all patients in this study 46% were males (Mean age 39.1) and 60% (mean age 29.8). The most common diagnosis was Tuberculosis (62%), Non Specific lymphadenitis (26%) and Malignancy (12%). Most common site of Lymphadenopathy is Cervical Lymphadenopathy (83.8%), Sub mandibular (6.4%), Axillary (6.4%), Supraclavicular (3.2%).

CONCLUSION:

This study concluded most common etiology of lymphadenopathy is tuberculosis and most commonly affected were females. AFB culture and sensitivity when compared to GeneXpert had 2.1 times more chances of detecting TB adenitis. HPE for TB adenitis when compared to GeneXpert had 3.8 times more chances of detecting TB adenitis.

KEY WORDS:

Lymph node, Tuberculosis, GeneXpert, AFB culture.

CONTENTS

INTRODUCTION

Body contains about 500 - 560 lymph nodes. Spleen , adenoids, Peyer patches and tonsils are the lymphatic tissue. The role is to neutralize the antigens entering into the body from the extra cellular fluid and the environment. Peripheral lymph nodes are located below the skin and palpable only if any pathological changes.

“Lymphadenopathy defines as a condition in which nodes are abnormal in size, texture and count. A lymph node is usually less than 10mm in size. The size varies in different locations, different ages and different size. An inguinal lymph node size upto 15 mm is normal. Epi trochlear node upto 5mm is normal. If the lymph node size more than 2cm needs clinical evaluation”.

It is important to take proper history taking and examination for diagnosing the etiology of the lymphadenopathy. The etiology varies in different areas. Tuberculosis is the commonest etiology for cervical lymphadenopathy in India and African countries. Most of the studies show common benign etiologies as Non specific reactive lymphadenopathy

AIMS & OBJECTIVES

1. To study the etiological causes that cause significant lymphadenopathy in adults patients referred to tertiary care hospital.

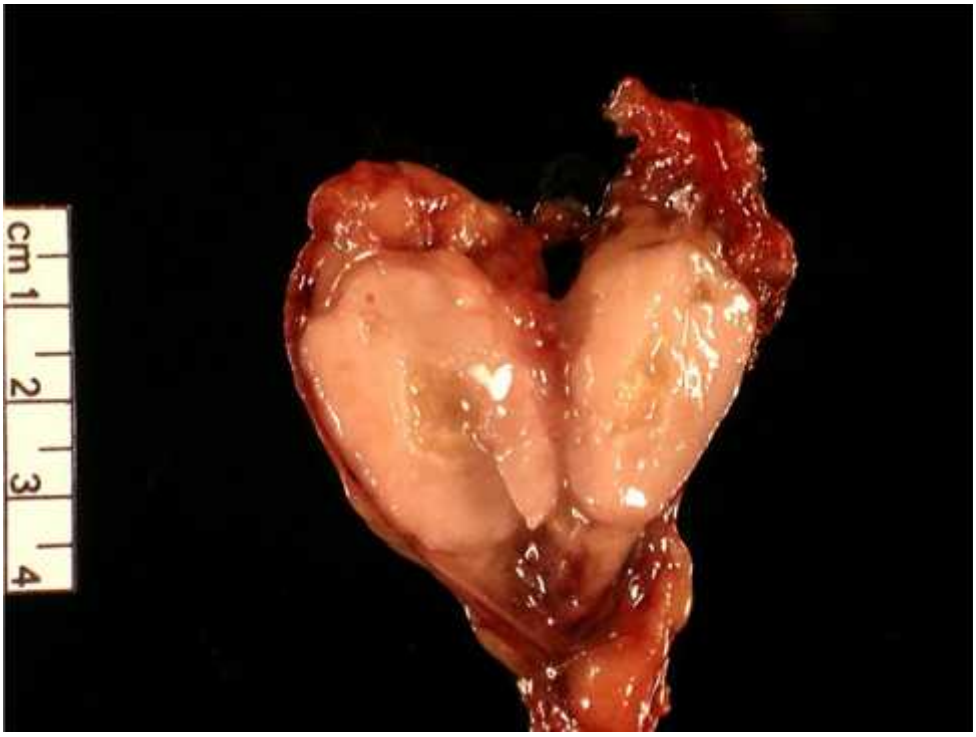
2. To describe the demography of patients referred with significant lymphadenopathy.

3. To describe the risk factors associated with significant lymphadenopathy.

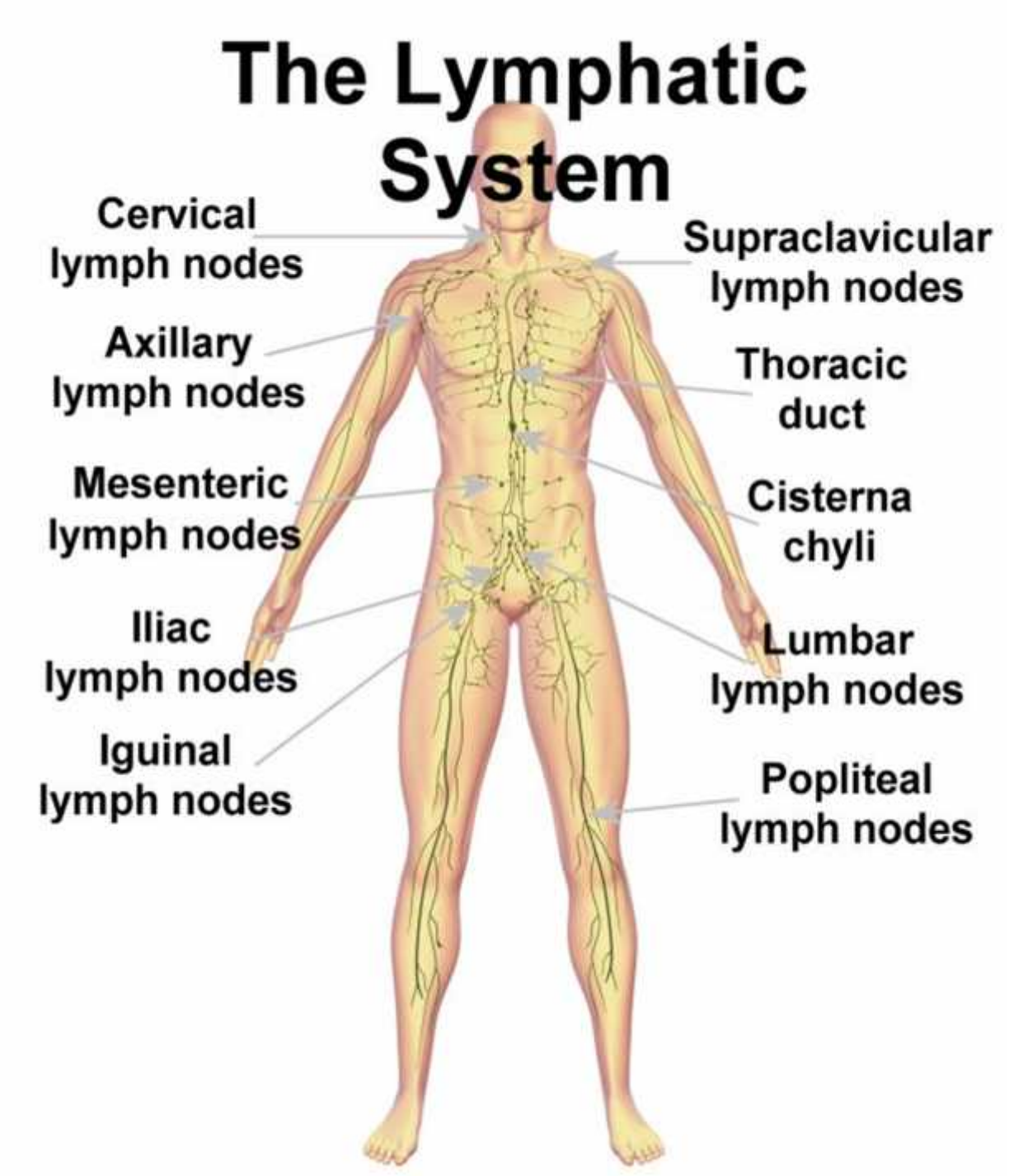
REVIEW OF LITERATURE

LYMPH NODE ANATOMY

"These are small oval or reniform bodies (0.1 – 2.5cm long), situated in the course of lymph vessels so that the lymph passes through them on its way to the blood. Generally each presents on one side a slight depression, termed as hilum through which blood vessels enter and leave the node. The efferent lymph vessels emerges from the node at hilum, afferent vessels enters at different parts of the periphery. The lymph node has two parts cellular part is called cortex and a darker medulla containing numerous cavities. These two part has indefinite line of demarcation".



“The cortex does not form the complete investment and its deficient at the hilum. The medulla reaches the surface of the node hence the efferent vessels are derived directly from medulla and afferent vessels empties into cortex”.



The lymph nodes are numerous in the thoracic mediastinum, posterior abdominal wall, in the abdominal mesenteries, pelvis, neck and proximal ends of the limbs.

AXILLARY LYMPH NODE:

The main lymphatic drainage of the upper limb is Axillary lymph node group. The axillary lymph nodes are scattered in the fibro fatty tissue of the axilla. They are divided into 5 groups

1. Anterior / Pectoral group
2. Posterior /Scapular group
3. Lateral group
4. Central group
5. Apical group

ANTERIOR GROUP:

It lies along the lower border of pectoral minor muscle accompanying lateral thoracic vessels. These nodes have direct contact with axillary tail of the breast . They receive lymphatic drainage from

1. Upper half of the anterior wall of trunk
2. Major part of the breast

POSTERIOR GROUP:

It lies along the subscapular vessels on the posterior axillary fold. They receive lymph from

1. Posterior wall of the upper half of the trunk
2. Axillary tail of the breast

LATERAL GROUP:

They lie along the upper part of the humerus medial to the axillary vein. They receive lymph from the upper limb

CENTRAL GROUP:

It lies on the fat of the upper axilla. They receive lymph from the preceeding lymph nodes and drain into apical node. They receive some direct vessel rom the floor of the axilla. It closely related to intercostobrachial nerve.

APICAL GROUP:

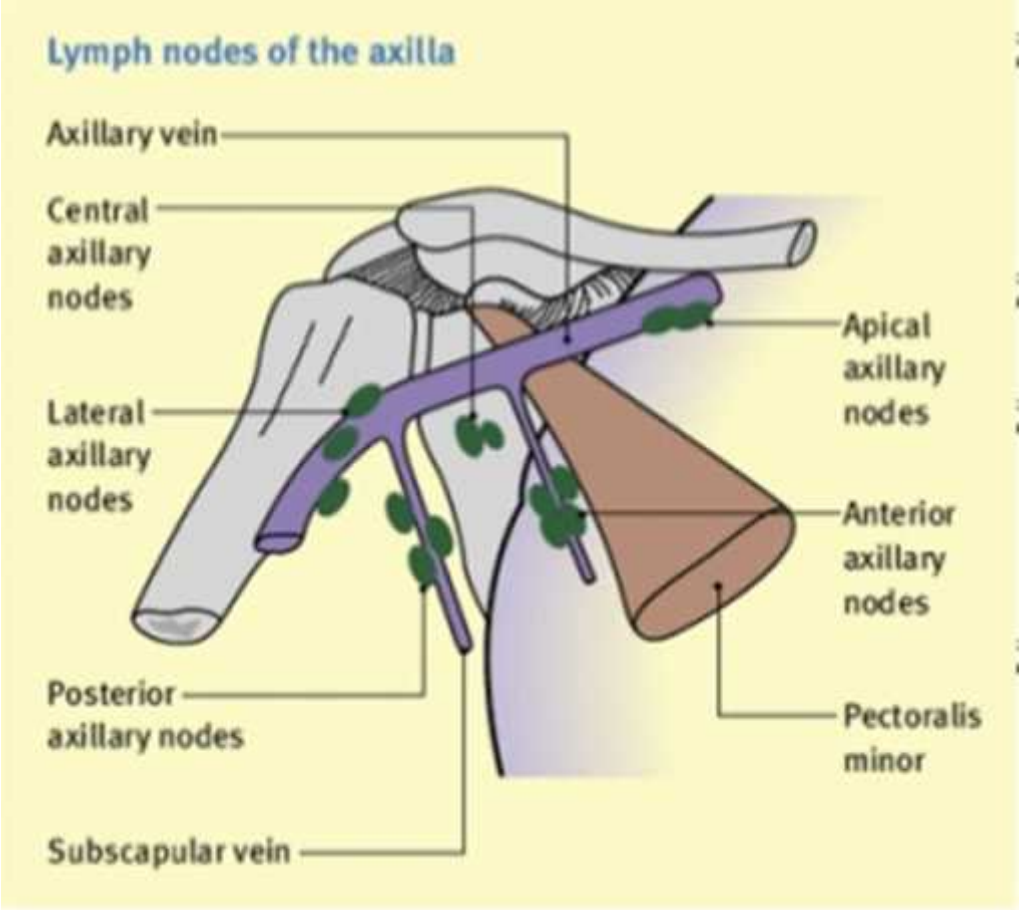
It lies deep to the clavipectoral fascia along the axillary vessels. They receive lymph from

1. Central group
2. Upper part of the breast
3. Thumb and its web space

The Lymphatic from the thumb accompanies the cephalic vein.

CLINICAL ANATOMY:

Axillary abscess should be incised in the floor of the axilla midway between the anterior and posterior axillary folds and nearer to the medial wall in order to avoid injury to the main vessels running along the anterior, posterior and lateral walls.



INFRA CLAVICULAR NODES:

Infra clavicular nodes lies on the clavipectoral fascia along the cephalic vein. They drains upper part of the breast and thumb with its web spaces.

DELTOPECTORAL NODES:

Lies on the deltopectoral group along the cephalic vein. It is the displaced node of the infraclavicular set and drains upper part of breast and thumb with its web spaces.

SUPRATROCHLEAR NODE:

Lies just above the medial epicondyle of the humerus along with basilica vein. It drains ulnar side of the hand and forearm.

LYMPH NODES OF HEAD AND NECK:

"The entire lymphatic drainage from the head and neck drains into deep cervical nodes either directly or through the peripheral nodes. The deep cervical nodes form the vertical chain situated along the entire length of the internal jugular vein".

JUGULO DIGASTRIC NODE:

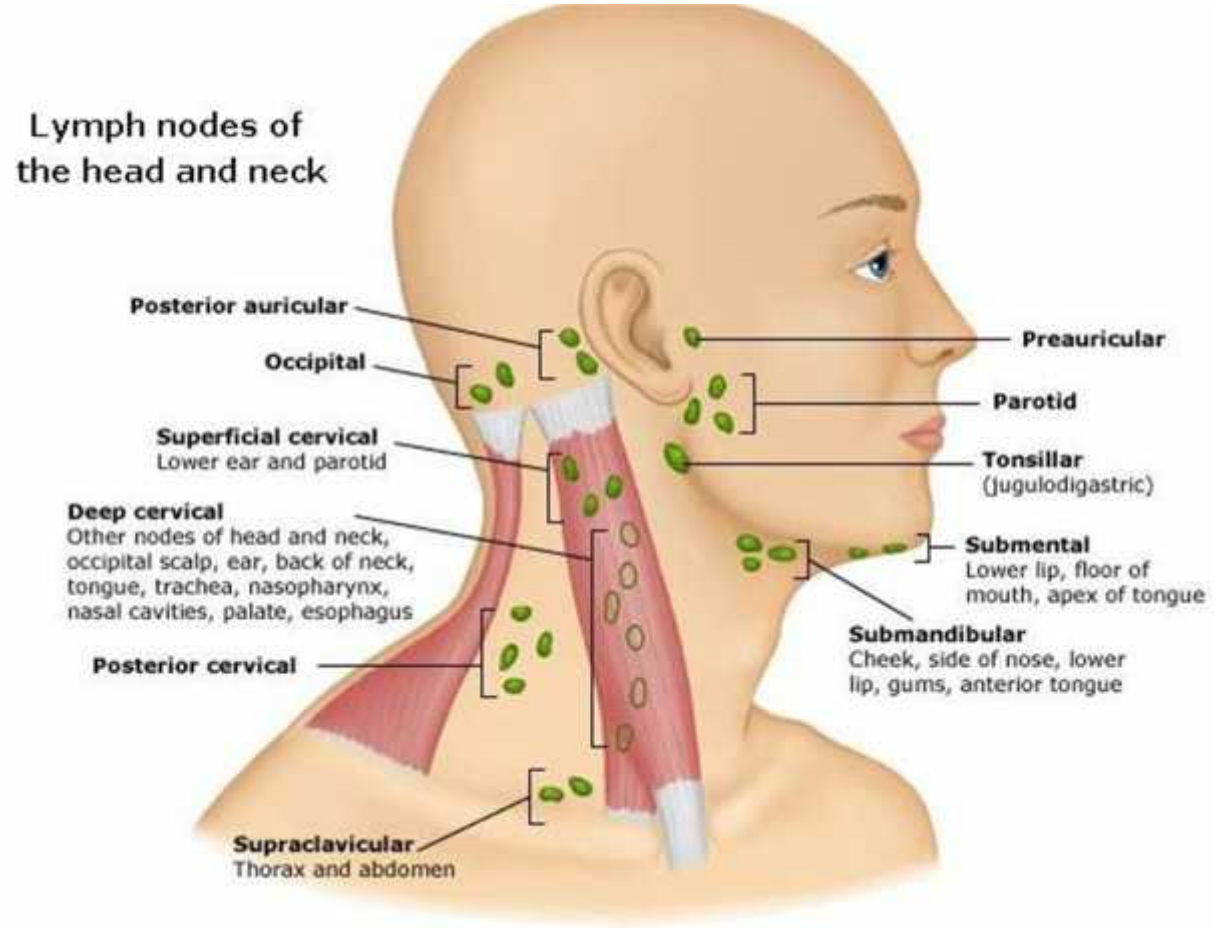
"It lies below the posterior belly of the digastrics between angle of mandible and anterior border of sternomastoid in the triangle bounded by posterior belly of digastric, facial vein and internaL jugular vein. It is the main node draining the tonsil".

JUGULO OMOHYOID NODE:

"Lies just above the intermediate tendon of the omohyoid under the cover of posterior border of sternomastoid. It is the main node draining the tongue".

LYMPHATIC TRUNK:

"Efferents of the deep cervical lymph nodes join together to form the jugular lymph trunks one on each side. The left jugular trunk opens into the thoracic duct. The right trunk may open either into the right lymphatic duct or directly into the angle of junction between internal jugular and subclavin veins".



PERIPHERAL CERVICAL NODES:

The peripheral nodes arranged in two circles.

1. Superficial circle
2. Deep circle

SUPERFICIAL CIRCLE OF CERVICAL LYMPH NODES:

1. Submental
2. Submandibular
3. Buccal and mandibular
4. Preauricular

5. Postauricular
6. Occipital
7. Anterior cervical
8. Superficial cervical

DEEP CIRCLE OF CERVICAL LYMPH NODES:

1. Pre laryngeal and re tracheal
2. Para tracheal
3. Retropharyngeal
4. Waldeyer's ring

SUPERFICIAL CIRCLE:

SUBMENTAL NODES:

Lies deep to the chin. Drains lymphatics from tip of the tongue and anterior part of floor of the mouth

SUBMANDIBULAR NODES:

Drains lateral surface of tongue, lower gum and teeth and central forehead

BUCCAL AND MANDIBULAR NODES:

"Buccal node lies on the buccinator and mandibular node at the lower of the mandible near the anteroinferior angle of the masseter in close relation to the mandibular branch of facial nerve".

Drains part of the cheek and the lower eyelid. Their efferents pass to the anterosuperior group of deep cervical nodes.

PREAURICULAR NODES:

Drain parotid gland , temporal region , middle ear

POST AURICULAR NODES:

"Post auricular nodes lies on the mastoid process superficial to sternomastoid and deep to auricularis posterior".

Drains strip of scalp just above and below the auricle, upper half of the medial surface and margin of the auricle and posterior wall of the external acoustic meatus. They are draining into postero superior group of deep cervical nodes.

OCCIPITAL NODES:

"Lies at the apex of the posterior triangle superficial to the attachment of the trapezius. They drain the occipital region of scalp".

Their efferents pass to supraclavicular group of postero inferior deep cervical nodes.

ANTERIOR CERVICAL NODES:

Lies along the anterior jugular vein. They drains the skin of the anterior part of the neck below the hyoid bone. They drains into deep cervical nodes of both sides.

SUPERFICIAL CERVICAL NODES:

Lies along the external jugular vein superficial to the sternomastoid muscle. They drains the lobule of the ear , floor of the external acoustic meatus , the skin over the lower parotid region and angle of the jaw. They finally drain into upper and lower deep cervical nodes.

DEEP CIRCLE:

PRE LARYNGEAL AND PRE TRACHEAL NODES:

It lies deep to the investing layer of deep cervical fascia. Pre laryngeal nodes lies on the cricothyroid membrane. Pre tracheal nodes lies infront of trachea below the isthmus of thyroid gland.

They drains larynx, trachea and isthmus of thyroid. They receive afferents from anterior cervical nodes. They finally drains into deep cervical nodes nearby

PARATRACHEAL NODES:

Lies on the side of the trachea and esophagus along with recurrent laryngeal nerve. They receive lymphatics from esophagus, trachea and larynx. They finally drain into deep cervical nodes.

RETROPHARYNGEAL NODES:

Lies infront of the prevertebral fascia and behind the bucco pharyngeal fascia. They extend laterally infront of the lateral mass of the atlas along with lateral border of longus capitis muscle.

They drains the pharynx , Eustachian tube , soft palate and posterior part of the hard palate and nose. They finally drain into upper deep cervical lymph nodes.

WALDEYER'S RING:

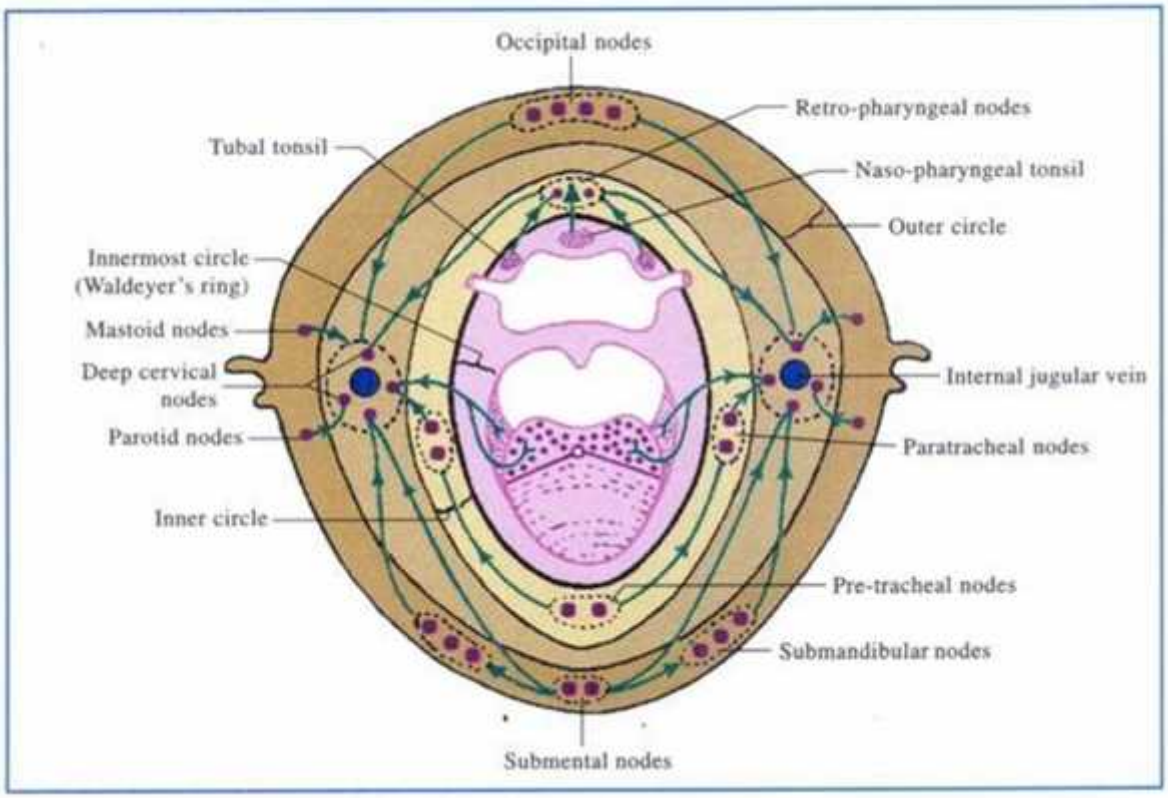
It is formed by the following lymphatic structures.

Lingual tonsil

Tubal tonsil

Palatine tonsil

Naso pharyngeal tonsil



LYMPHATIC DRAINAGE OF LOWER LIMB:

It is classified into superficial and deep system.

SUPERFICIAL SYSTEM:

Superficial inguinal lymph nodes

DEEP SYSTEM:

1. Deep inguinal nodes
2. Popliteal nodes
3. Anterior tibial nodes

SUPERFICIAL INGUINAL NODES:

It is very important because it drains the skin and fascia of the lower limb, perineum and the trunk below the umbilicus.

They are divided into three groups.

1. Lower vertical group
2. Upper lateral group
3. Upper medial group

Lower vertical group is placed along with the terminal part of great saphenous vein. It contains 405 nodes. It drains skin and fascia of the lower limb and the short saphenous territory except the gluteal region.

Upper lateral group node is situated below the lateral part of inguinal ligament. It contains about two or three nodes. It drains the skin and fascia of the upper part of the lateral side of thigh, gluteal region, the flank and back below the level of umbilicus.

Upper medial group is placed below the medial end of the inguinal ligament. It contains one or two nodes along with superficial epigastric vessels. It drains anterior abdominal wall below the level of umbilicus, perineum, genitalia except glans penis, anal canal below the pectinate line, vagina below the hymen, supero lateral angle of uterus and penile part of male urethra.

All superficial inguinal lymph nodes drain into deep inguinal nodes piercing cribriform fascia. Some may directly drains into external iliac nodes.

DEEP INGUINAL NODES:

It lies medial to the upper part of the femoral vein . it is about 4-5 in number. The most proximal part of this group of node is called gland of Cloquet or Rossenmuller.

Rossenmuller lies in the femoral canal. These nodes receive the lymphatic drainage from superficial inguinal nodes, popliteal nodes, glans penis or clitoris and deep lymphatics of the lower limb along with femoral vessels.

They finally drains into external iliac nodes.

POPLITEAL LYMPH NODES:

This group of nodes lies near the termination of short saphenous vein and deep to deep fascia. They receive lymphatic from territory of the short saphenous vein, deep part of the legs and knee joint.

Their efferent run along the popliteal and femoral vessels and terminate in the deep inguinal nodes.

ANTERIOR TIBIAL NODES:

It lies along with upper part of anterior tibial artery. It receives lymphatics from the anterior compartment of the leg

MICROSCOPIC ANATOMY:

A lymph node consists of a continuous framework which includes a capsule, trabecular and reticular fibres, and the cells entangled in this framework. The capsule is composed mainly of collagen fibres, a few fibroblasts and some elastin fibres.

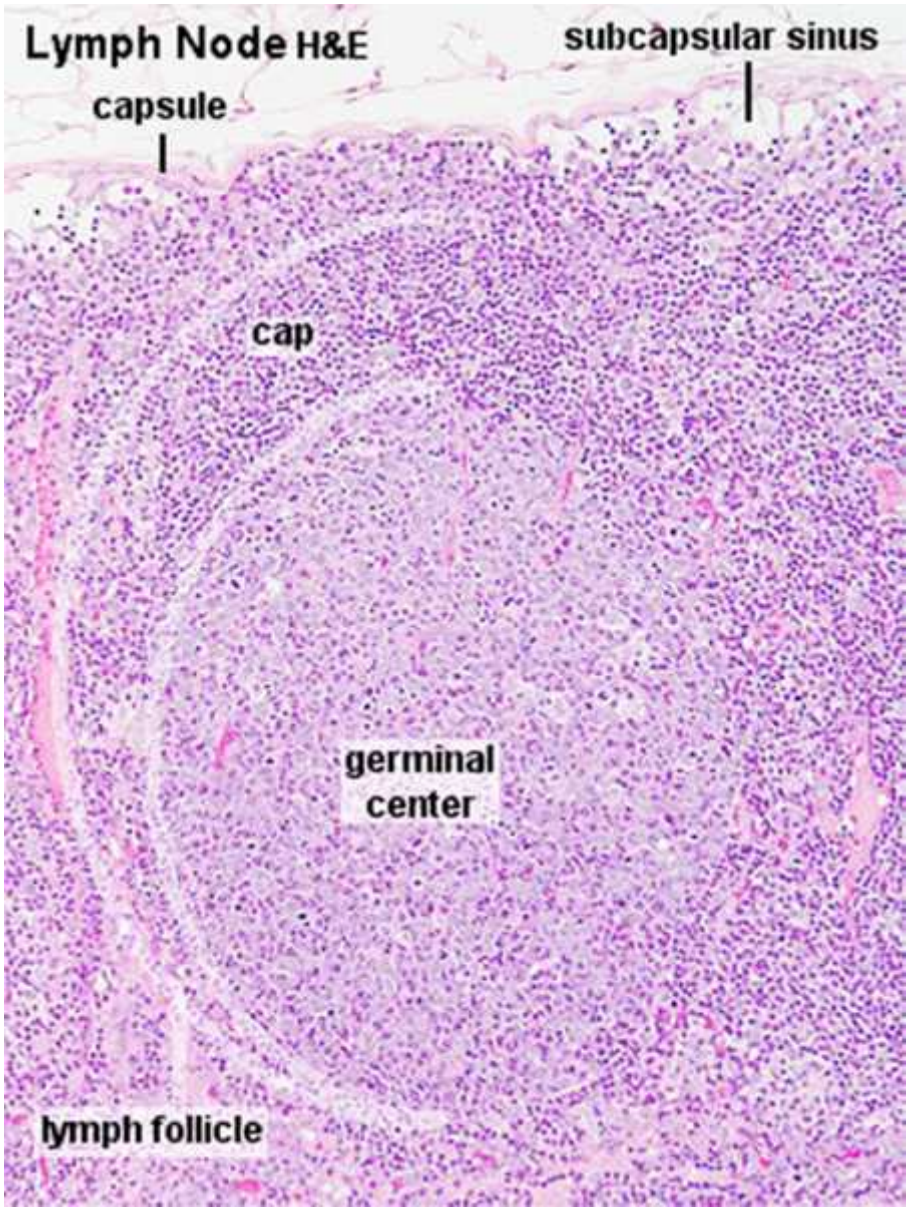
The capsule surrounds the outside of the node and from its deep surface, trabeculae of the similar structure extend radially into the inner side of the node, where they were continue with the reticulum that forms the lymphoid tissue.

The reticulum is the meshwork of fine reticulin fibres and attendant cells which permeates the spaces outlined by the capsule and trabeculae providing mechanical support for the cell masses lying in their vicinity.

Lymph nodes are permeated Lymphatic channels through which the lymph percolates after it has entered from the afferent lymphatic vessels.

“The arrangement of lymphatic vessels ensures the maximum exposure of the lymph to the macrophages and lymphocytes which lines them and are

entangled between the fibres cross them. The afferent vessels enters at different points on the periphery of the node".



"After entering they are branched and forming a dense plexus in the capsule, open into the subcapsular sinus. From this area numerous radial cortical sinuses lead towards the medulla, eventually coalescing to form the larger

medullary sinuses which are confluent at the hilum with the efferent lymphatics or vessels draining the node".

These spaces are everywhere lined by endothelial cells and there is a constant passage of lymphocytes, macrophages and other cells through the sinus walls in the both directions.

LYMPHATIC BLOOD SYSTEM:

Arteries and veins supply the lymph nodes enter through the hilum and give off branches which are traverse in the medulla accompanying connective tissue trabeculae and give off few minor branches

On reaching the cortex arteries divide into arterioles and capillaries which form numerous anastomosing loops eventually to pass back into the highly branched venules and veins. The capillaries are profusely around the periphery of the follicles and fewer in the germinal centre.

Post capillary venules are abundant in the para cortical zones where they are an important route of lymphocyte migration. The pattern of vascularization is considerably altered when the node undergo antigenic stimulation to produce large number of lymphocytes.

The structure of blood vessel is usual except post capillary venules which are lined by cuboidal endothelial cells. In between the cuboidal endothelial cells

colloid material pass quite readily into the perivascular space and allows extensive movement of lymphocytes from the blood stream into the para cortex area of the lymph node and probably also in the reverse direction.

In addition to the vessels of the nodal medulla some vessels leave the lymph node through its principal trabeculae and general capsule supplying the surrounding connective tissues.

Majority of the B and T lymphocytes particularly in the sinuses and around the germinal centres. The distribution of the lymphocytes is different in the various parts of lymph node. In the cortex the cells are densely packed and form the isolated masses called lymphatic follicles (nodules). The number and degree of the lymphatic follicles vary from time to time according to the level of antigenic stimulation .

The central part of the each follicle is composed of larger cells and deeply staining and dividing more rapidly than those at the periphery. The central areas are called the germinal centre and contains mainly lymphoblasts which are divided mitotically produce small lymphocytes which are accumulated initially in the marginal zones around the germinal centre.

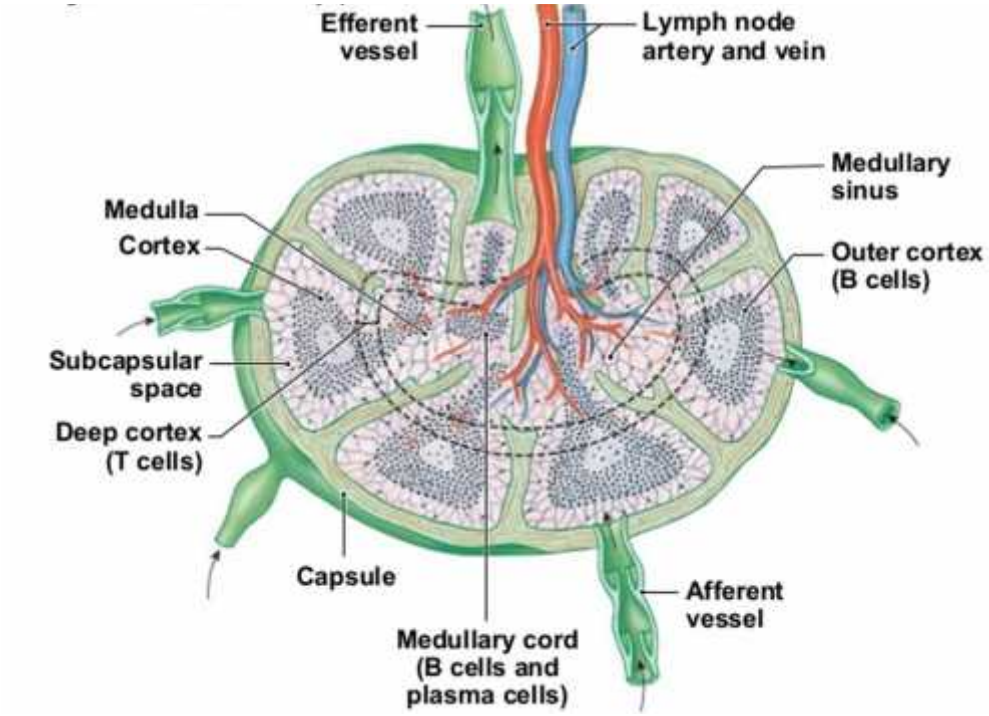
“They leave the peripheral part of the follicles to enter the lymphatic sinuses which convey them through the medulla to the efferent lymph vessel at the hilum

of the node. In the medulla of the node, the lymphocyte are loosely packed than they are in the cortex".

"Under conditions of intense antigenic stimulation, when the node drains a focus of infection the whole node increases in size and vascularity and called as reactive node. The number and size of its germinal centre increases with raised lymphopoiesis, macrophage proliferation and plasma cell differentiation within the sinuses".

CELL ZONES IN LYMPH NODES:

Lymph node cortex is divided into number of indistinct lobules by connective tissue trabeculae passing inwards from the capsule and within these lobules lies lymphoid follicles. It is divided into 3 zones.



Zone 1 is a region of loosely packed cells consisting largely of small lymphocytes ,macrophages and plasma cells.

Zone 2 is more densely packed within zone 1, limited to cortical and para cortical areas of nodes consisting of mainly small lymphocytes and macrophages.

Zone 3 contains the germinal centres forming the core of the lymphoid follicles and they are mostly in antigenically stimulated lymph nodes. Zone 3 include large lymphoblasts undergoing mitosis together with macrophages.

Fibroblasts , reticulin fibres and associated cells are scattered throughout all 3 zones.

Non lymphocytic cell types in lymph nodes:

1.	Endothelial cells
2.	Fibroblasts
3.	Macrophages
4.	Perivascular cell including pericytes
5.	Dendritic cells

FUNCTIONS OF LYMPHOID SYSTEM:

- Provision of an intricate network of spaces of large volume and surface area through which lymph circulates slowly.
- Phagocytic action of macrophage
- Trapping of antigens by the phagocytes
- Provisional centre for lymphocyte production and pool of stem cells potentially capable of transforming into antibody producing B cells and mature T cells
- Interaction between antigen laden phagocyte and lymphocyte to mount an immune response both cellular and humoral
- Portal entry of blood borne lymphocytes back into the lymphatic sinus
- Humoral antibody production

EMBRYOLOGY:

Initial stage of development of lymphatic system according to two views. –

First view (Huntington 1908; McClure and Bulter 1925), lymphatic spaces commences as clefts in the mesenchyme and lined by endothelium. These spaces forms capillary plexus from which lymph sacs are derived.

Second view (Sabin 1902) – the earliest lymph vessels arise as a capillary off shoot from the endothelium of the veins and which form capillary plexus; later these plexus lose their connections with the venous system and become confluent to form lymph sacs.

In human embryo lymph sacs from which lymph vessels are derived are six in number.

- Two paired – (jugular and posterior lymph sac)
- Two unpaired – (Retroperitoneal and cistern chyli)

The position of the sacs as follows

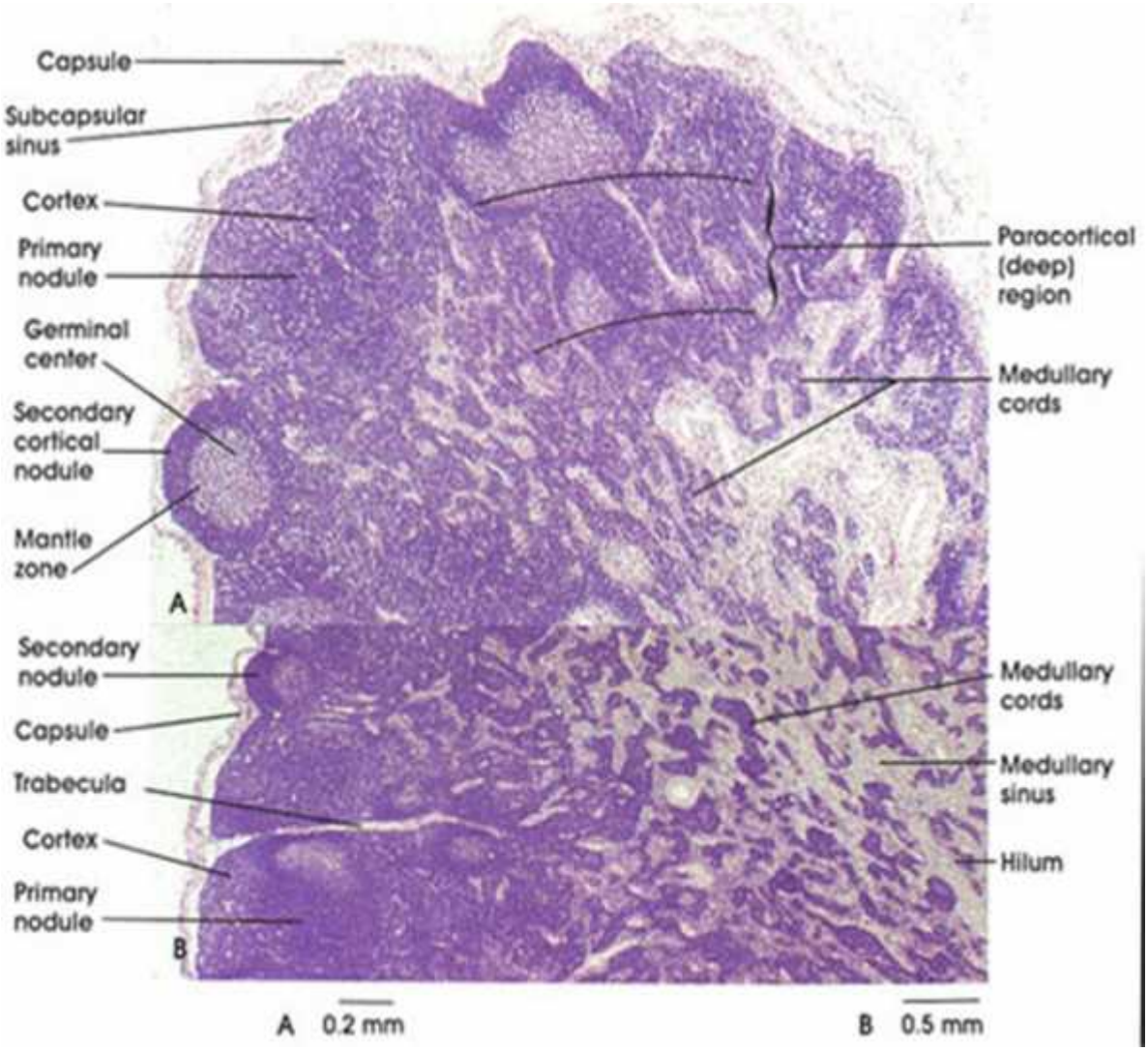
1. Jugular – first appear , at the junction of subclavian and precardinal vein
2. Posterior sac – at the junction of the iliac vein with the postcardinal vein
3. Retroperitoneal – at the root of the mesentry near suprarenal glands
4. Cistern chyli – opposite to the third and fourth lumbar vertebra

The lymph vessels are budding out from the lymph sacs along the lines of corresponding course of the embryonic vessels. The thoracic duct is phylogenetically bilateral structure. Numerous valves are lied down the duct during the fifth month of intra uterine life but many of them disappear before birth.

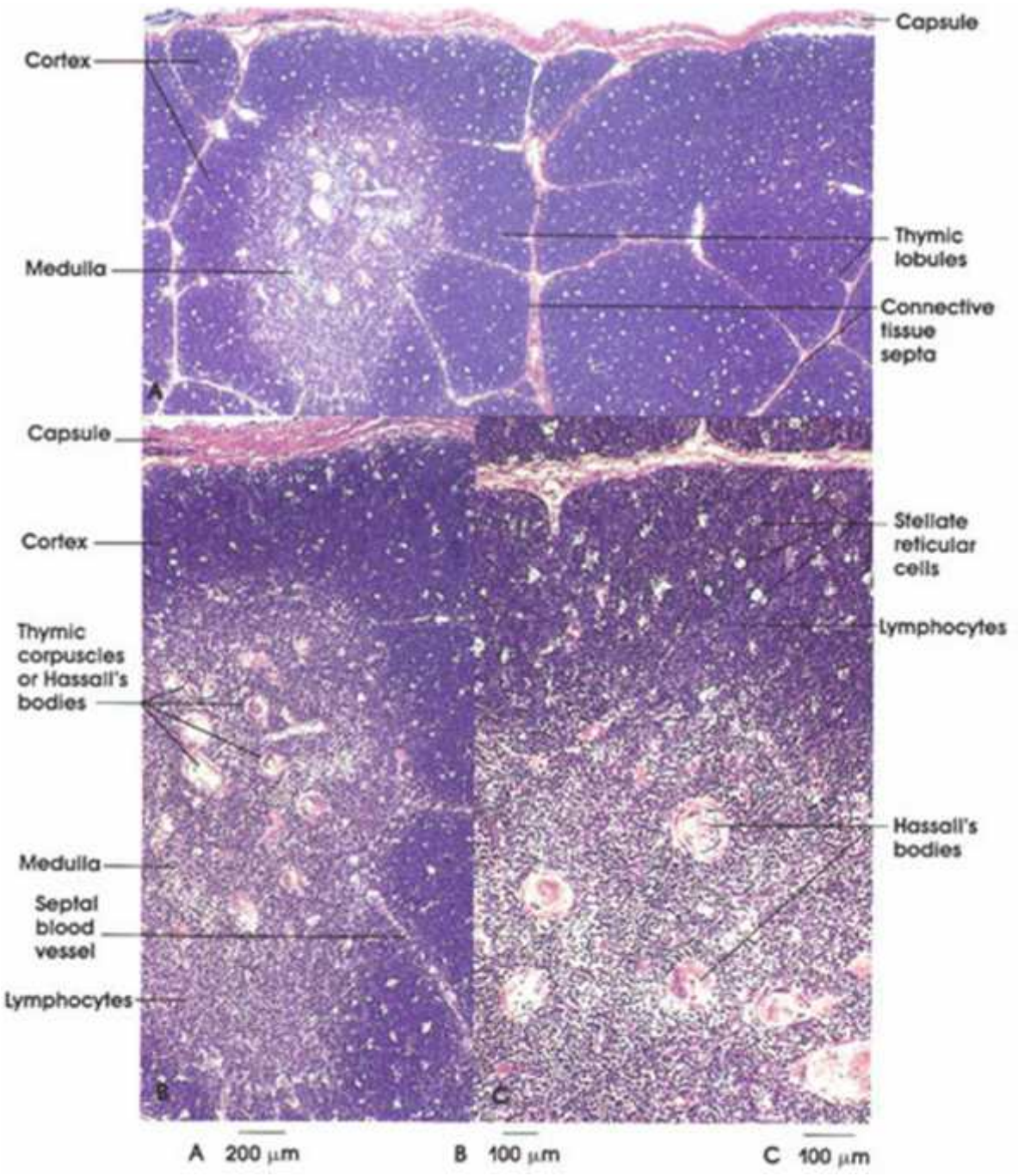
All lymphatic sacs except the cistern chili are divided into numerous slender connective tissue bridges at the later stages. Lymph sacs are invaded by lymphocytes and are transformed into groups of lymph node. The lymph sinuses representing the portions of the original cavity of the sac.

Haemel lymph nodes are said to develop as mesenchymal condensation in close relation to blood vessels rather than lymphatics.

HISTOLOGY



Section shows cortex , capsule ,germinal centre , secondary cortical nodules , medullary cords and medullary sinus.



Section shows medulla , septal blood vessels , Hassall bodies and lymphocytes.

CLASSIFICATION OF MYCOBACTERIA:

TUBERCLE BACILLI	Human – M.tuberculosis Bovine – M.bovis Murine – M.microti Avian – M.avian Cold blooded – M.marinum
LEPRA BACILLI	Human – M.leprae Murine – M.leprae murium
MYCOBACTERIA CAUSING SKIN ULCER	M.ulcerans M.balnei
ATYPICAL MYCOBACTERIA	Photo chromogens Scoto chromogens Non photo chromogens Rapid growers
JOHNE'S BACILLI	M.paratuberculosis
SAPROPHYTIC MYCOBACTERIA	M.butyricum M.phlei M.stercoris

MYCOBACTERIA:

Mycobacteria means fungus like bacteria. They do not stain readily. Resist discolouration with dilute mineral acids and hence called as ‘Acid fast bacilli’.They are aerobic, non motile, non capsulated and non sporing.

First member of this genus was identified was lepra bacilli, discovered by Hansen in 1868. Koch’s (1882) isolated a mammalian tubercle bacilli and proved it causative role in tuberculosis by satisfying Koch’s postulates.

MYCOBACTERIA TUBERCULOSIS

MORPHOLOGY:

It is straight or slightly curved rod measuring about 3 microns , occurring singly or pairs or as small clumps. The size depends on conditions of growth and long filamentous, club shaped and branching forms may be seen.

When stained with carbol fushin by ziehl neelson method or by fluorescence dyes, they resist decolourisation by 20% sulphuric acid and absolute alcohol for 10 minutes.

CULTURE CHARACTERISTICS:

The colonies grow slowly; generation time in vitro about 14-15 hours; Growth appears in about two weeks and may sometimes takes upto eight weeks. Optimum temperature is 37-40^{0}C . Optimum pH is 6.4-7.0. It is an obligate aerobe. It grows luxuriantly in culture and termed as eugenic. The addition of 0.5% glycerol improves the growth of M.tuberculosis.

Human tubercle bacilli does not grow in the presence of P-nitrobenzoic acid .They do not have exacting growth requirements but are highly susceptible even to traces of toxic substances like fatty acids in culture media.

The toxicity is neutralized by serum albumin or charcoal.

SOLID MEDIA	**LIQUID MEDIA**
LJ medium	Dubos
Petragnini	Middlebrook
Dorset egg	Proskauer
Tarshis	Beck
Loeffler Serum	Sula's and Sauton
Pawlowsky	

Liquid medias are usually not used for the cultivation but are used sensitivity testing, chemical analysis and preparation of antigens and vaccines.

On solid media M. tuberculosis forms dry, rough, raised, irregular colonies with a wrinkled surface. They are creamy white, becoming yellowish or buff coloured on further incubation. They are tenacious and not easily emulsified.

In liquid media without dispersing agents the growth begins at the bottom, creeps up the sides and forms a prominent surface pellicle which may extend along sides above the medium. Diffuse growth is obtained in Dubos medium containing Tween – 80.

BIO CHEMICAL REACTIONS:

Several tests has been described for identification of Mycobacterium species.

1. Niacin test
2. Aryl sulphatase test
3. Neutral red test
4. Catalase –peroxidase test
5. Amidase tests
6. Nitrate reduction test

PATHOGENESIS:

"In un-exposed, immunocompetant person CMI against Mycobacterium, results in immunity to infection to the bacteria and develop hypersensitivity to mycobacterial antigens. The histologic presentation of tuberculosis show caseating granulomas and then cavitation. These are the manifest of the hypersensitivity that develops in concordance with the host immune protection".

"The effector cells that mediate immune protection also contribute to hypersensitivity and so results in tissue destruction. Appearance of hypersensitivity in tuberculin skin test show immunity to infection of the organism".

Macrophages are the main cells infected by M. tuberculosis. Tuberculosis bacilli replicate essentially unchecked initially. Later the CMI stimulates macrophages to stop the proliferation of the bacteria.

M. tuberculosis enters macrophages by endocytosis by the following

1) mannose receptors bind lipoarabinomannan

2) a glycolipid in the bacterial cell wall and

3) complement receptors bind opsonized mycobacteria

“Tuberculosis organisms replicate within the phagosome by inhibiting fusion of the phagosome and lysosome. Tuberculosis inhibits phagolysosome formation by blocking Ca^{2+} signals and recruit the macrophages secrete group of proteins that mediate phagosome-lysosome fusion. In the earliest stage of primary tuberculosis (<3 weeks) in the nonsensitized persons, bacteria multiply in the pulmonary alveolar macrophages and airspaces, causing bacteremia and disseminating the mycobacterium to many sites. Asymptomatic to mild febrile illness is the presentation”.

The genetic component of the individual may alter the course of the tuberculosis. Polymorphisms in the NRAMP1 gene, results in progress of the disease due to the absence of immune response. NRAMP1 is a transmembrane protein in endosomes and lysosomes that pushes the divalent cations out of the lysosome. NRAMP1 may block microbial growth by reducing availability of ions for the bacteria.

“After 21 days of infection, a T-helper 1 (T_H1) action is mounted to kill the mycobacterium. The mycobacterial antigens that enter draining lymph nodes stimulates the T cells. Differentiation of T_H1 cells depends on IL-12, which is produced by antigen-presenting cells that have encountered the mycobacteria. M. tuberculosis secretes ligands for TLR2, and initiation of TLR2 by these ligands results in production of IL-12 by dendritic cells”.

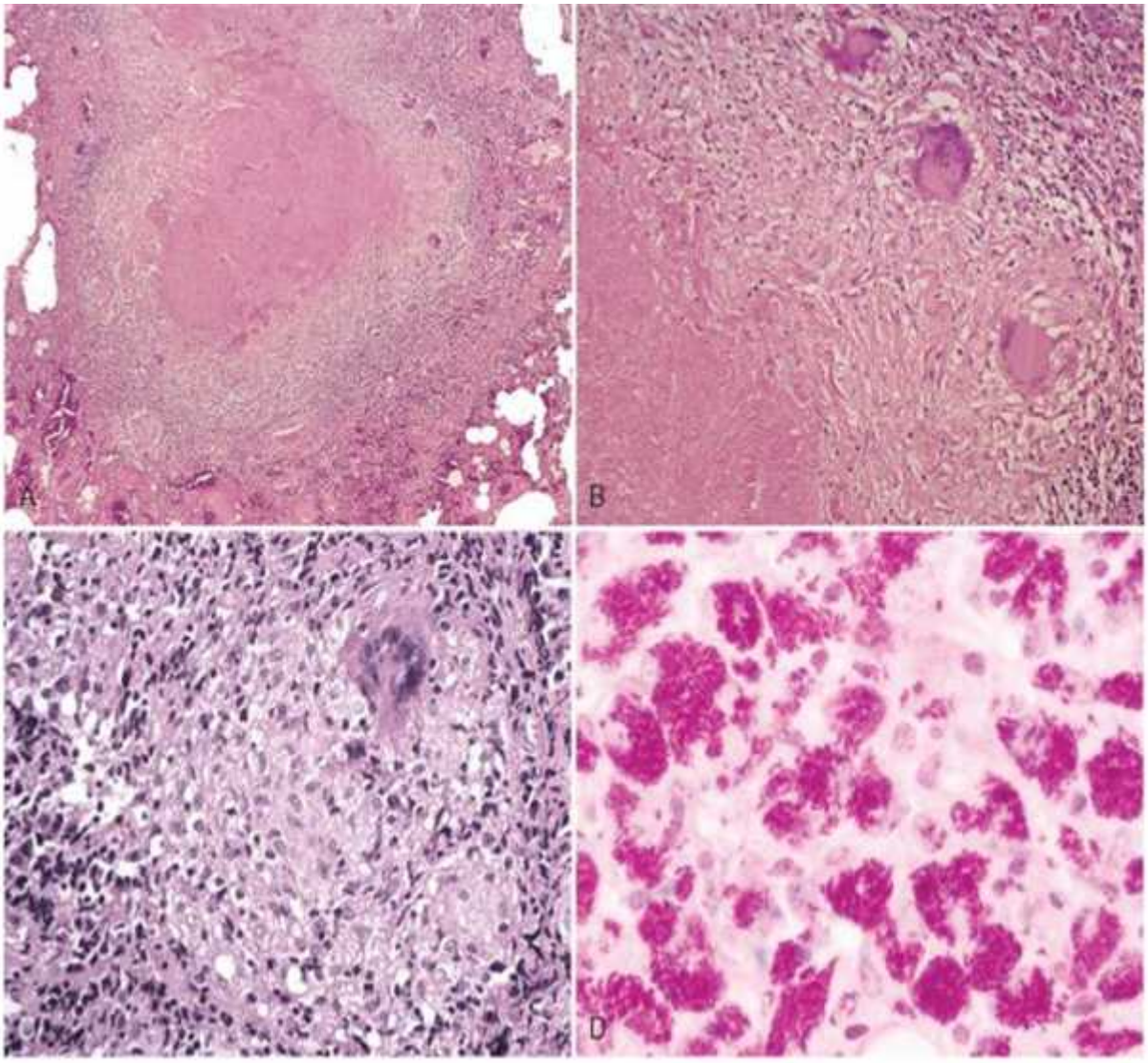
"T_H1 cells, in lymph nodes and in the lung, release IFN-γ. INF-γ is the mediator that enables macrophages to inhibit the M. tuberculosis. IFN-γ stimulates phagolysosome in infected macrophages, exposing the bacteria to acidic environment. IFN-γ also stimulates nitric oxide synthase, which produces nitric oxide, capable of neutralizing mycobacterium cell wall".

"The T_H1 response results in the formation of granulomas with caseous necrosis. Macrophages differentiate into the "epithelioid histiocytes" by activated by IFN-γ that result in the granulomas and fuse to form giant cells. In most cases this halts the infection before tissue destruction or illness. In some the infection progresses due to old age or immunosuppression, and ongoing immune response results in tissue destruction due to caseation and cavitation. Macrophages also produce TNF, which promotes more monocytes".

"NK-T cells recognize mycobacterial lipid antigens attached to CD1 on antigen-presenting cells, or T cells that show a γδ T-cell receptor, also form IFN-γ. T_H1 cells have a central role in this process. Defects in any of the steps in forming T_H1 response result in disease worsening state".

In summary, immunity to *M.* tuberculosis is primarily mediated by T_H1 cells, which stimulate macrophages to kill the bacteria. This immune response, while largely effective, comes at the cost of hypersensitivity and accompanying tissue destruction. Reactivation of the infection or re-exposure to the bacilli in a

previously sensitized host results in rapid mobilization of a defensive reaction but also increased tissue necrosis. Just as hypersensitivity and resistance are correlated, so, too, the loss of hypersensitivity (indicated by tuberculin negativity in a previously tuberculin-positive individual) may be an ominous sign that resistance to the organism has faded



Tubercle granuloma at histology

“Tubercle at low magnification (**A**) and in detail (**B**) show central caseation with a ring of epithelioid along with multinucleated giant cells. This is the normal response in patients with cell-mediated immunity to the organism. Not all tubercular granulomas might have central caseation (**C**); hence, special stains for AFB must be done when granulomas are seen. In immune-compromised without cellular immunity layers of fatty macrophages are seen that are filled with mycobacteria (demonstrable with AFB stains)”.

EPIDEMIOLOGY:

In India and tropical countries TB is the commonest cause for lymphadenopathy in adults. Next is malignancy. In patients with HIV in recent days increased incidence of extra pulmonary TB is associated with more than 50%.

In general less than 1% of patients having lymphadenopathy is due to malignant diseases. In children leukemia , in adolescents often due due Hodgkin disease.

Hodgkin disease is common after 10 years of age and male more affected in childhood. The combination with HIV , EBV infection is increased risk for Hodgkin’s disease in economically poor countries and low socio economic status. In worldwide NHL is the 4^{th} common malignancy in males.

HISTORY TAKING:

History taking is necessary to determine the cause of lymphadenopathy.

Age, sex, onset, duration of symptoms, underlying co morbidities are important. In addition history of exposure to pets, ingestion of drugs and foods, history of recurrent infection and immunodeficiency contributes to the diagnosis.

Chewing of tobacco, smoking, alcohol and UV radiation increases the risk of the metastatic malignancies of head & neck and organs. Immune deficient individuals like HIV positive can have Kaposis sarcoma and Non Hodgkin lymphoma.

Family history of malignancy can raise the suspicion of Breast carcinoma, melanoma and dysplastic nevus syndrome. Lymphadenopathy lasting less than 1 month to 12 months without increase in size have less chances to be a malignant.

SIGNS AND SYMPTOMS:

MONO NUCLEOSIS LIKE SYMPTOMS:

High fever

Pharyngitis

Tender lymphadenopathy

Splenomegaly

All above are present in Infectious mononucleosis and also in CMV infection, Toxoplasmosis, HIV, HHV type I .In HIV generalized lymphadenopathy is seen after 2-6 weeks of exposure to HIV virus.

History of travel to an endemic area or contact with TB infected persons may lead to painless progressive gradually increase in size of lymphadenopathy. The lymph node may be single or matted. It may be suggestive of tuberculosis infection.

Lymphadenopathy associated with arthralgia , muscle weakness , rashes and anemia suggestive of auto immune diseases like Rheumatoid arthritis, SLE etc.

B symptoms :

Fever

Night sweats

Weight loss more than 10% in 6 months

Suggestive of lymphoproliferative malignant disorders. Generalized pruritis, painful lymphadenopathy following alcohol intake may suggestive of Hodgkin disease. Petechiae , purpura, lymphadenopathy and splenomegaly may present in acute leukemias.

EXAMINATION OF LYMPHADENOPATHY:

All patients of lymphadenopathy must be examined thoroughly. Examination of lymph nodes for its location, size, consistency and tenderness is important.

LOCATION:

1. Generalized
2. Localized
3. Presence of red lymphangitic streaks
4. Supra clavicular area is high risk for malignancy

SIZE:

Palpable lymphadenopathy of more than 0.5 cm in supraclavicular, epitrochlear, cervical, popliteal and iliac nodes ; and inguinal node of more than 1.5 cm is suggestive of pathological

PAIN AND TENDERNESS:

It may be due to infection and also induced by hemorrhage in the neoplastic nodes or rapid tumour expansion.

CONSISTENCY:

Chronic inflammation leads to fibrosis make the node hard in palpation.

Acute inflammation due to tension on the capsule causes tenderness.

Stony hard and painless node is usually metastatic or granuloma of lymph node.

Firm and rubbery nodes are seen in lymphoma. Matted lymph nodes are seen in mycobacterium infection.

MOBILITY:

Lymphadenopathy due to infections and collagen vascular diseases are freely mobile in subcutaneous plane. Rubbery mobile nodes usually suggestive of lymphoma. Hard fixed node is usually due to metastatic and malignancy diseases.

ASSOCIATED SYMPTOMS:

Splenomegaly associated with lymphadenopathy seen in infectious mono nucleosis, acute lymphoma, Hodgkin disease , NHL and sarcoidosis.

Skin lesions are common in malignancies such as melanoma. Traumatic lesions can be the cause for axillary lymphanopathy by the infections in the draining area.

The benign reactive inguinal lyphadenopathy seen in bare foot walkers.

Localized lymphadenopathy is typical of infections and due to sexually transmitted diseases like HSV ,Gonococcal infection, syphilis, LGV ,and Granuloma inguinale

Malignancy is rare when only inguinal nodes are enlarged. Carcinoma of penis, vulva and anus can involve inguinal lymphadenopathy.

PERIPHERAL LYMPHADENOPATHY – DIFFERENTIAL DIAGNOSIS:

LOCALISED PERIPHERAL LYMPHADENOPATHY:

Cervical	Viral: URI, Infectious mononucleosis, Human herpes virus/ CMV/ HIV, Coxsackie virus Bacterial: staphylococccus aureus, Streptococcal pyogenes (Gr A),

	Mycobacterium Malignancy: Hodgkin, NHL, CA thyroid, metastatic carcinoma.
Supraclavicular	Abdominal and thoracic malignancy CA breast, Mycobacterial and fungal infections
Axillary	Bacterial: Staphylococccus aureus, Streptococcal pyogenes (Gr A), Mycobacterium Malignancy : Lymphoma, Leukemia
Inguinal	Benign reactive lymphadenopathy STD infections Lymphoma, carcinoma of penis and vulva, metastatic melanoma

GENERALISED PERIPHERAL LYMPHADENOPATHY:

Infections	Infectious mononucleosis, HIV, Miliary TB, Typhoid fever, syphilis, Plague
Malignancy	Lymphoma and AML
Auto immune disorders	SLE, RA, Sjogren syndrome, Sarcoidosis
Drug reactions	Phenytoin, Allopurinol, Atenolol
Lipid storage diseases	Gaucher's & Niemann Pick disease

LABORATORY DIAGNOSIS:

1. Complete hemogram
2. Viral markers – HIV, HBV, HCV, CMV
3. Anti nuclear antibody
4. ESR
5. Mono spot test
6. VDRL
7. Mantoux test
8. Peripheral smear
9. Imaging – Colour Doppler USG, CT scan and MRI scan
10. Chest X Ray
11. FNAC (USG guided)
12. Gram staining and special staining of FNAC of lymph nodes
13. Sputum AFB and C/S
14. GeneXpert analysis
15. TB C/S
16. Histopathological examination

GENEXPERT:

The Xpert MTB/RIF detects DNA sequences specific for Mycobacterium tuberculosis and rifampicin resistance by polymerase chain reaction. It is based on the Cepheid GeneXpert system.it is a rapid and simple-to-use nucleic acid amplification tests (NAAT). The Xpert MTB/RIF purifies and concentrates Mycobacterium tuberculosis bacilli from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR.

The process identifies all the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the Mycobacterium tuberculosis genome in a real time format using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very little technical training required to operate.

A review to assess the diagnostic accuracy of Xpert TB found that when used as an initial test to replace smear microscopy it had pooled sensitivity of 88% and specificity of 98% . However when Xpert TB was used as an add-on for cases of negative smear microscopy the sensitivity was only 67% and specificity 98%.

In a clinical study conducted the sensitivity of the MTB/RIF test on just 1 sputum sample was 92.2% for culture-positive TB; 98.2% for smear- and culture-

positive cases; and 72.5% for smear-negative, culture-positive cases, with a specificity of 99.2%. Sensitivity and higher specificity were slightly higher when 3 samples were tested

New TB tests are needed because of the difficulties associated with the tests that are currently used both to diagnose TB as well as to detect drug resistance.

Traditionally TB has been diagnosed by looking for evidence of TB bacteria either through the use of the chest X-ray, through sputum smear microscopy, or through the culturing of bacteria. Each of these TB tests has their disadvantages, one of the most significant for culture being the time that it takes and for sputum the matter of accuracy.

In addition to diagnosing TB there is also a need to test for drug resistance, in order that the most effective TB treatment can be provided. Culture is currently the main tool for drug susceptibility testing. However, not only is the length of time it takes a problem, but it also requires trained personnel and expensive laboratory equipment.

The Xpert MTB/RIF has been developed by the Foundation for Innovative New Diagnostics (FIND), who have partnered with the Cepheid corporation and the University of Medicine and Dentistry of New Jersey. Some of the funding for the development of the Xpert MTB/RIF was provided by the NIH

WHO recommended that the test should be used as the initial diagnosis test in individuals suspected of having MDR TB, or HIV associated TB. They also suggested that it could be used as a follow on test to microscopy in settings where MDR TB and/or HIV is of lesser concern, especially in smear negative specimens, because of the lack of accuracy of smear microscopy. They did however say that they recognised the major resource implications of using it in this second way.

WHO did also emphasize that the test does not eliminate the need for conventional microscopy culture and drug sensitivity testing, as these are still required to monitor treatment progress and to detect other types of drug resistance.

The Xpert MTB/RIF cannot be used for treatment monitoring, as it detects both live and dead bacteria.Further guidance was subsequently provided by WHO on the rapid implementation of the new test

Disadvantages

There are a number of disadvantages which include:

- The shelf life of the cartridges is only 18 months;
- A very stable electricity supply is required;
- The instrument needs to be recalibrated annually;
- The cost of the test;
- The temperature ceiling is critical.

Advantages

The main advantages of the test are, for diagnosis, reliability when compared to sputum microscopy and the speed of getting the result when compared with culture. For diagnosis of TB, although sputum microscopy is both quick and cheap, it is often unreliable.

It is particularly unreliable when people are HIV positive. Although culture gives a definitive diagnosis, to get the result usually takes weeks rather than the hours of the Xpert test.The main advantage in respect of identifying rifampicin

resistance, is again the matter of speed. Normally to get any drug resistance result takes weeks rather than hours.

FALGON TUBE:

Suitable for cell centrifugation applications such as pelleting and separation by density gradients; molecular biology applications including concentrating bacteria for DNA isolation, purification and precipitation of nucleic acids, sample storage (ambient temperature from -4 to +60°C) and centrifugation of precipitates.

- Dark blue graduations in 0.5mL increments are visible with clear or turbid samples
- Solvent-resistant white writing area
- Sterilized by gamma irradiation, nonpyrogenic
- Supplied blue HDPE 20mm threaded dome seal closures provide positive seal over the full circumference

Clarified Polypropylene

- Resistant to alcohols and mild organic solvents (not recommended for extraction procedures)
- Translucent wall for easy viewing
- Stable from -4° to +60°C (24.8° to 140°F)
- (Withstand centrifugal forces to 12,000 x g RCF

Polystyrene

- Optically clear for easy viewing of sample
- Stable from 4° to 60°C (39° to 140°F)
- Withstand centrifugal forces up to 1800 x g RCF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CULTURE MEDIA:

“The definite diagnosis of tuberculosis demands that M.tuberculosis be recovered on culture media and identified using differential in vitro tests. Many different media have been devised for cultivating tubercle bacilli and three main groups can be identified, viz egg-based media, agar-based media and liquid media.”

“The ideal medium for isolation of tubercle bacilli should (a) be economical and simple to prepare from readily available ingredients, (b) inhibit the growth of contaminants, (c) support luxuriant growth of small numbers of bacilli and (d) permit preliminary differentiation of isolates on the basis of colony morphology.”

“For the culture of sputum specimens, egg-based media are the first choice, since they meet all these requirements.”

PREPARATION OF LOWENSTEIN-JENSEN MEDIUM:

“Lowenstein-Jensen (LJ) medium is most widely used for tuberculosis culture. LJ medium containing glycerol favours the growth of M.tuberculosis while LJ medium without glycerol but containing pyruvate encourages the growth of M. bovis.”

“Ingredients:”

“Mineral salt solution with malachite green	
Potassium dihydrogen phosphate anhydrous (KH2PO4)	2.4g
Magnesium sulphate anhydrous	0.24g
Magnesium citrate	0.6g
Asparagine	3.6g
Glycerol (reagent grade)	12ml
Malachite green, 2% solution	20ml
Malachite green solution 2%	
Malachite green dye	2.0g
Distilled water	100ml”

“Dissolve the dye in distilled water completely. Filter and store in refrigerator.Dissolve the ingredients in order in about 300ml distilled water by heating. Add glycerol, malachite green solution and make up 600ml with distilled water.”

“This solution should be sterilized by autoclaving at 121o C (15 psi) for 30 minutes. Cool to room temperature. If required, this solution may be stored in the refrigerator.”

“Homogenised whole eggs :Fresh country hen’s eggs those are not more than seven days old, are cleaned by scrubbing thoroughly with a hand brush in water and soap. Let the eggs soak for 30 minutes in soap solution. Rinse eggs thoroughly in running water and soak them in 70% ethanol for 15 minutes. Before handling the clean dry eggs scrub and wash the hands with a disinfectant. Crack the eggs with the edge of the beaker into a sterile flask and beat them in a sterile blender for 30 seconds to one minute.”

“Preparation of complete medium:

The following ingredients are aseptically pooled in a large, sterile flask and mixed well:

Mineral salt solution with malachite green	600ml
Homogenised eggs (25-30 eggs, depending on size)	1000ml

The complete egg medium is distributed in 6-8ml volumes in sterile universal containers and the caps tightly closed and inspissated without delay to prevent sedimentation of heavier ingredients.”

“Coagulation of medium:

Before loading, heat the inspissator to 85o C to quicken the build-up of the temperature. Place the bottles in a slanted position in the inspissator and coagulate the medium for 50 minutes at 85o C (since the medium has been prepared with sterile precautions this heating is to solidify the medium, not to sterilise it.”

"The quality of egg media deteriorates when coagulation is done at too high a temperature or for too long. Discolouration of the coagulated medium may be due to excessive temperature. The appearance of little holes or bubbles on the surface of the medium also indicates faulty coagulation procedures."

"Sterility check:"

"After inspissation, the whole media batch of the media bottles should be incubated at 35^0 C- 37^0C for 24 hours as a check for bacterial sterility. After 24 hours 5% of the slopes should picked up randomly and continued for incubation for 14 days to check for fungal sterility. In both the cases the contamination rate should not be > 10 %."

"Storage:"

"The LJ medium should be dated and stored with the batch number in the refrigerator and can keep for upto 4 weeks if the caps are tightly closed to prevent drying of the medium."

Note :

• "Prepare one batch of plain LJ media one week before the anticipated arrival of samples (one batch is 1620 ml of LJ fluid sufficient for 324 LJ slopes). The requirement is two LJ slopes for each specimen."

"One batch of media provides 20 sets for drug susceptibility testing and an additional 44 plain LJ slopes. One set of drug susceptibility testing media consists

of 5 plain LJ slopes, 2 slopes each for four drugs and one PNB containing LJ media."

"In the initial period, it is recommended not to prepare more than one batch of media per day."

"L-J Medium with sodium pyruvate:"

"For the cultivation of M. bovis, LJ medium is enriched with 0.5% sodium pyruvate. In the preparation of the mineral salt solution, glycerol is omitted and 8.0g sodium pyruvate is added for every 600 ml. This is added to 1 litre of egg fluid, mixed well and distributed."

"DRUG SUSCEPTIBILITY TESTS :"

"Drug susceptibility testing is one of the most difficult procedures to perform and standardize in the mycobacteriology laboratory. Proficiency in susceptibility tests demands an understanding of:"

"• The origin of drug resistance

• The variation in stability of drugs subjected to different conditions of filtration, heat or storage

• The alteration in the activity of certain drugs when incorporated into different kinds of media

• The type of susceptibility test performed

• The reading and reporting of test results

• The criteria of resistance .”

“Drug susceptibility tests should be performed in the following instances:

• For relapse or re-treatment cases

• To change the drug regimen when drug resistance is suspected

• Undertaking drug resistance surveillance studies in a region/country.”

“There are three general methods used for determining drug susceptibility of mycobacterium: the proportion method, absolute concentration method (MIC method) and the resistance ratio method, and When properly standardized and performed, all three methods have been shown to be equally satisfactory.”

“In India, under RNTCP, proportion method is advised since large numbers of laboratories have standardized this method for DST.”

METHODS :

“THE PROPORTION METHOD:”

“It enables precise estimation of the proportion of mutants resistant to a given drug. Several 10-fold dilutions of inoculum are planted on to both control and drug –containing media; at least one dilution should yield isolated countable (50 -100) colonies.”

“When these numbers are corrected by multiplying by the dilution of inoculum used, the total number of viable colonies observed on the control medium, and the number of mutant colonies resistant to the drug concentrations tested may be determined. The proportion of bacilli resistant to a given drug is then determined by expressing the resistant portion as a percentage of the total population tested.”

“THE ABSOLUTE CONCENTRATION METHOD:”

“This method uses a standardized inoculum grown on drug-free media and media containing graded concentrations of the drug(s) to be tested. Several concentrations of each drug are tested, and resistance is expressed in terms of the lowest concentration of the drug that inhibits growth; i.e., minimal inhibitory concentration (MIC). This method is greatly affected by inoculum size and the viability of the organisms.”

“THE RESISTANCE RATIO METHOD:”

“It compares the resistance of unknown strains of tubercle bacilli with that of a standard laboratory strain. Parallel sets of media, containing twofold dilutions of the drug, are inoculated with a standard inoculum prepared from both the unknown and standard strains of tubercle bacilli. Resistance is expressed as the ratio of the minimal inhibitory concentration (MIC) of the test strain divided by the MIC for the standard strain in the same set.”

“The proportion method is currently the method of choice and the simple version of bacterial suspension and interpretation of results are given below.”

“The economic variant of proportion method used under RNTCP.”

“PROCEDURE DETAILS FOR THE PROPORTION METHOD STANDARD ECONOMIC VARIANT BACTERIAL SUSPENSION:”

“Inoculum:”

“With a loop, a representative sample of approximately 4-5 mg is taken from the primary culture and placed in a McCartney bottle containing 1 ml sterile distilled water(SDW) and 6 glass beads of diameter 3 mm. The bottle is vortexed for 20–30 seconds; 4-5 ml of distilled water is added slowly under continuous shaking.”

"Allow the coarse particles to settle down. Decant the mycobacteria carefully into another clear, sterile McCartney bottle. The opacity of the bacterial suspension is then adjusted by the addition of distilled water to obtain a concentration of 1 mg/ml of tubercle bacilli by matching with McFarland standard No.1"

<u>"Preparation of suspensions for economic variant of proportion method:"</u>

"1 ml SDW with six 3 mm glass beads + 1 loopfull (3 mm internal diameter) of culture vortexed for 20 – 30 seconds

↓

Add 4 ml of SDW to the above

↓

Adjusted turbidity with McFarland 0.05 with SDW.

↓

S2-10 –2 2 loopfull of neat + 2ml of SDW

↓

S4-10-4 2 loopful of S2 + 2ml of SDW"

“Precautions:”

“- Avoid touching the media while picking the colonies,

- Cool down the loop sufficiently before picking the colonies,

- Try to take loopful of colonies in one sweep, by touching all colonies on the LJ slope.

- Avoid touching the water of condensation while scrapping the colonies

- Emulsify the initial inoculums onto the walls of the McCartney bottle”

“PREPARATION OF MC FARLAN NEPHELOMETER BARIUM SULFATE STANDARD No.1 (Paik, G. 1980)”

“1. Prepare 1% aqueous barium chloride and 1% sulphuric acid (AR) solutions. (100 mg of Barium chloride (anhydrous) in 10 ml of SDW and 0.1 ml of sulphuric acid (AR) in 10 ml of SDW).

2. Add 0.1 ml of 1% Barium Chloride to 9.9 ml of 1% Sulphuric acid to obtain the McFarland standard No.1, which matches with 1 mg/ ml of M. tuberculosis.

3. Seal the tube with parafilm and label as McFarland standard No.1 with date of preparation.”

“During preparation of neat bacterial suspension, the comparison is done against a white background. After preparing the standard Neat suspension, either a loop or pipettes may be used for further processing and for inoculation. It is recommended to use loop method under RNTCP.”

“ Inoculation for DST – Loop method”

“The loop should be of Nichrome wire (24 SWG) and should have an internal diameter of 3 mm, which delivers 0.01 ml. Delivery volume must be verified by weighing 10 loopfuls of distilled water deposited on a filter paper.”

“The two bacterial dilutions required for inoculation with the loop are 10–2 and 10–4 from the neat prepared as above. The dilutions 10-2 is produced by discharging two loopfuls of the bacterial suspension, standardized at 1 mg/ml, into a Bijou bottle containing 2 ml of distilled water. Mix the contents by shaking.”

“Similarly, the dilution 10–4 is produced by discharging two loopfuls of the 10–2 dilution into a small tube containing 2 ml of distilled water. Mix the contents by shaking. Two slopes of medium without drug and one slope of medium with drug for each of the four drugs are inoculated with a loopful of each dilution.”

“Precautions:”

“Avoid touching the water of condensation while inoculation. Don’t allow water condensation to spread over the media surface

- Inoculate uniform suspension in all the slopes

The standard strain M. tuberculosis, H37Rv is tested with each new batch of medium. The recommended drug concentrations are 4 mg/l for streptomycin, 0.2 mg/l for isoniazid, 40 mg/l for rifampicin and 2 mg/l for ethambutol.”

“ Additional recommendation:”

“The inoculum indicated above usually provides satisfactory countable colonies for the test, i.e., more than 10 and less than 100 colonies per control slope seeded with smaller inoculum. However, in some laboratories, the yield may prove smaller, owing to different growth conditions. In such cases, initially, the labs should seed 10^{-2}, 10^{-3} and 10^{-4} dilutions.”

“INCUBATION AND READING”

“Incubate the slopes at 37^{o}C.

Read the proportion tests at 28 days and again at 42 days.”

“Record growth as

3+	Confluent growth
2+	More than 100 colonies
1-99 cols	Actual number of colonies

"When the number of colonies on a given dilution is less than 15, count the number of colonies with the next larger inoculum, or estimate if more than 100. (Make no attempt to estimate the number of colonies if the growth is 3+)"

"INTERPRETATION OF TESTS:"

"1. First reading is taken at 28th day after inoculation.

2. Count the colonies only on the slopes seeded with the inoculum that has produced exact readable counts or actual counts (up to 100 colonies on the slope). This inoculum may be the same for the control slopes and the drug-containing slopes, or it may be the low inoculum (10–4 dilution) for the control slopes and the high inoculum (10–2 dilution) for the drug-containing slopes.

3. The average number of colonies obtained for the drug-containing slopes indicates the number of resistant bacilli contained in the inoculum.

4. Dividing the number of colonies in drug containing slopes by that in drug free slopes gives the proportion of resistant bacilli existing in the strain. Below a certain

value – the critical proportion – the strain is classified as sensitive; above that value, it is classified as resistant. The proportions are reported as percentages.

5. If, according to the criteria indicated below, the result of the reading made on the 28th day is “resistant”, no further reading of the test for that drug is required: the strain is classified as resistant. If the result at the 28th day is “sensitive”, a second reading is made on the 42nd day only for the sensitive strain. The final definitive results for all the four drugs should be reported on 42nd day. If the strain is resistant for all the four drugs on 28th day, then the report can be given on the same day. Otherwise, incomplete reports should not be given before 42nd day.

6. In case growth on the control media is poor even after six weeks (i.e., few or no colonies on the 10-4 bacterial dilution), the test should be repeated.

7. Repeat the test: Test showing an average of less than 10 colonies in the control slopes seeded with the smaller inoculum.”

MATERIALS AND METHODS

- All Patients who fit with the inclusion criteria and give consent will undergo the test.
- Demographic details collected.
- Anaesthetist fitness.
- Risk consent.
- GA/RA/LA
- Gene X PERT in Falgon test tube
- Biospy – one in formalin for HPE and another in sterile container for AFB culture.
- Results tabulated and studied.

SUBJECT SELECTION

INCLUSION CRITERIA:

- Age > 18 yrs
- Patient should have significant lymphadenopathy which is described as
- **Size:** Insignificant if < 2cm
- In axilla and inguinal, insignificant if < 3cm
- In the supraclaviclar fossa > 1cm is significant
- **Consistency:** soft (insignificant), rubbery (classically lymphoma) , hard (classically malignancy & granulomatous infection).
- **Tender** (classically infection) vs. **non-tender** (classically malignancy)

EXCLUSION CRITERIA:

- Age < 18 yrs
- Any seriously ill patient
- Unfit for anaesthesia
- Patient 2-12 years old commonly present with insignificant lymph nodes in neck secondary to frequent viral infection.

Study period : December 2014 –AUGUST 2015

Sample size : 50

Study design : Prospective and Retrospective

Study procedures :

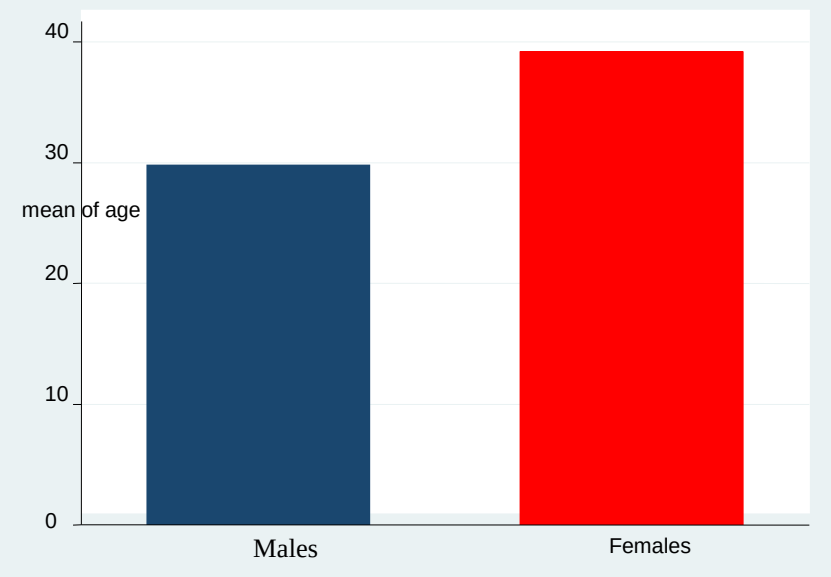
All eligible patients were informed about the study procedures in detail. Informed consent was obtained from willing patients. After getting anaesthetist fitness, biopsy of the concerned lymph node was done. The sample was sent for three investigational procedures namely geneXpert, histo pathological examination and mycobacterial culture and drug susceptibility testing.

Statistical analysis

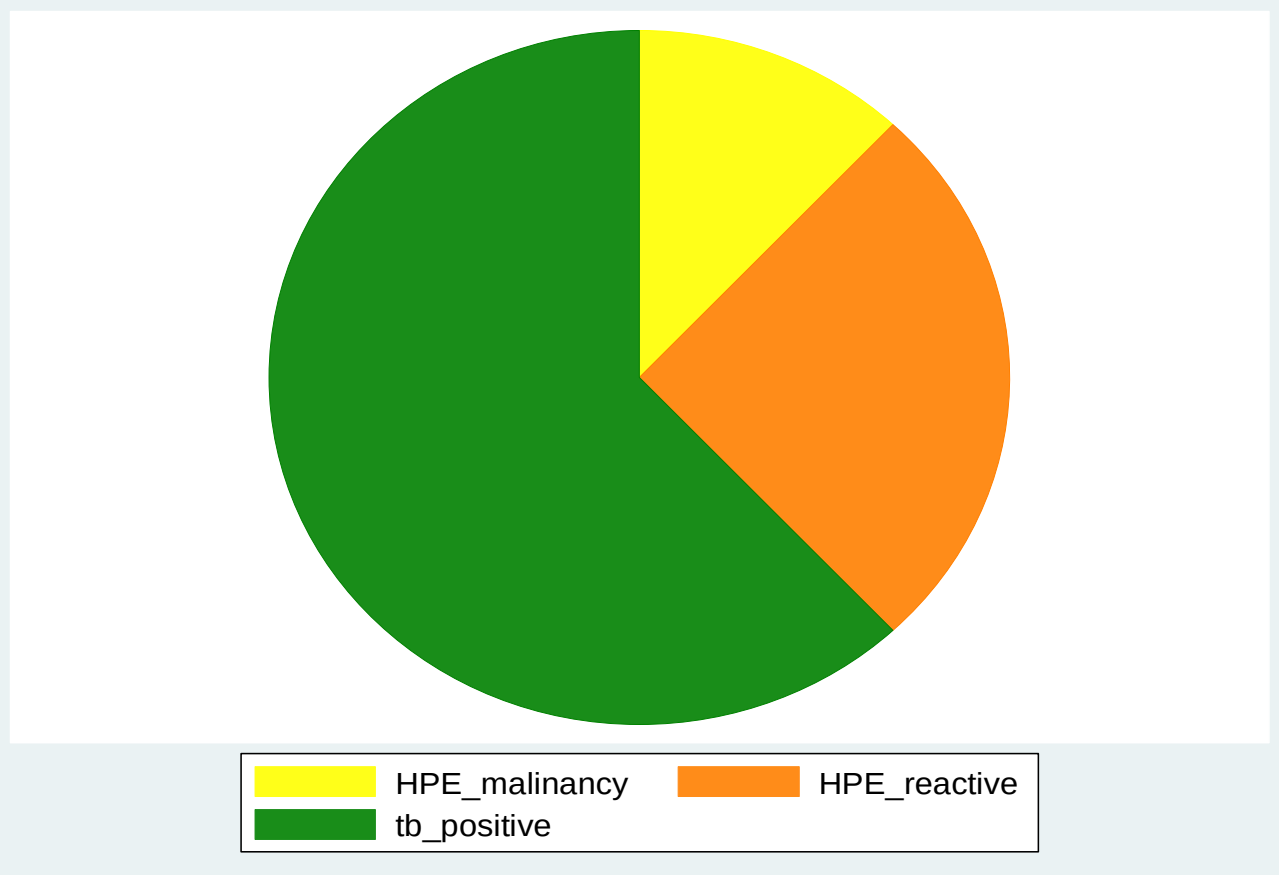
The data was entered in Excel and analysed in STATA 16. Descriptive statistics was used to summarise the individual characteristics.

DATA ANALYSIS AND RESULTS

From Dec 2014 to August 2015, total of 50 patients were recruited. Among them, 40% were males and60% were females. The mean age of the participants was 33.5 years (95% CI: 29.2-37.9). The mean age among females was 29.8 years (95% CI: 25-34.5) and the mean age among males was 39.1 years (95% CI: 31.4-46.9). The figure below shows the age distribution among male and female participants.



With histo pathological examination, 31 (62%) patients had histo pathological changes suggestive of TB. Malignant changes were seen in 6 (12%) patients. Non specific reactive lymphadenitis was seen in 13 (26%) patients. The figure below shows the different histology changes reported in the participants.



The table below shows the sex distribution with histo pathological changes in the lymph nodes.

Index	Frequency
TB_positive	
Female	22
Male	9
HPE_Malignancy	
Female	3
Male	3
HPE_Reactive Node	
Female	5
Male	8

The distribution of lymph node enlargement among the patients is as follows.The cervical lymph node enlargement was seen in majority of patients (83.87%), followed by submandibular lymph nodes (6.45%), axillary nodes (6.45%) and supra clavicular nodes (3.23%).

TB Positive	FREQUENCY	PERCENTAGE
SUB MANDIBULAR	2	6.45
SUPRA-CLAVICULAR	1	3.23
AXLLARY	2	6.45
CERVICAL	26	83.87

Among the 50 participants, 26 patients were positive for Mycobacterial culture for tuberculosis, 16 patients positive for M.tuberculosis with GeneXpert and 31 patients positive in histo pathological examination. GeneXpert and culture was both positive for M.TB in 13 patients. HPE and culture was positive in 23 patients. HPE and geneXpert was positive for M.TB in 17 patients. The table below summarises the results positive for M.TB in HPE, AFB culture and geneXpert.

TB POSITIVE	
Culture for AFB positive	26
Gene expert positive	17
Gene expert & AFB culture positive	13
HPE TB ADENITIS	31
HPE & AFB CULTURE POSITIVE	26
HPE & GENE EXPERT	16

The odds ratio was calculated for HPE, AFB culture and sensitivity and GeneXpert test by Fisher Exact test.

Fisher exact test for HPE and GeneXpert

HPE_TB	Gene_expert_positive 0	1	Total
0	18	1	19
	2.4	4.6	7.0
1	15	16	31
	1.5	2.8	4.3
Total	33	17	50
	3.8	7.4	11.3

Fisher's exact = 0.001
1-sided Fisher's exact = 0.001

Odds ratio was calculated for diagnosis of TB by GeneXpert compared to HPE. had 3.8 times more chances of diagnosing TB adenitis than GeneXpert with P-value of 0.001.

Fisher exact test for AFB culture and sensitivity and GeneXpert

AFB_c/s	Gene_expert_positive 0	1	Total
0	20	4	24
	1.1	2.1	3.2
1	13	13	26
	1.0	2.0	3.0
Total	33	17	50
	2.1	4.1	6.2

Fisher's exact = 0.018
1-sided Fisher's exact = 0.013

Odds ratio was calculated for diagnosis of TB by GeneXpert compared to AFB culture and sensitivity. AFB culture and sensitivity had 2.1 times more chances of diagnosing TB adenitis than GeneXpert with P-value of 0.018.

DISCUSSION & CONCLUSION

This study describes the etiological causes of lymphadenopathy in patients attending tertiary care centre among south Indian population. The following were the results of the study

a. Among the study participants, 60% were females and they presented at younger age compared to males.

b. Histo pathological changes suggestive of TB was seen in 62% of the subjects.

c. Malignant changes were seen in 12% of the subjects.

d. Non specific reactive lymph adenitis was seen in 26% of patients.

e. The most common site of lymphadenopathy was cervical region

f. Among patients diagnosed with TB lymphadenitis, 71% were females.

g. All the 31 patients diagnosed with TB lymphadenitis were positive for M.TB in histo-pathological examination.

h. 17 samples were positive with GeneXpert and 26 samples were positive for AFB culture.

i. AFB culture and sensitivity when compared to GeneXpert had 2.1 times more chances of detecting TB adenitis.

j. HPE for TB adenitis when compared to GeneXpert had 3.8 times more chances of detecting TB adenitis.

The fact that TB lymphadenitis is more common among females is supported by other studies done by Kulkarni and Frimodt Moller 1969, Krishnaswami et al 1972 and Pamra 1974.

Cervical region is the most common site of lymphadenopathy in this study which is also established in other studies by Doctor 1964, Frase 1965 and Kent 1967. More evidence is needed to establish the role of GeneXpert in diagnosis of lymph node tuberculosis.

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PATIENT PROFORMA

PATIENT DETAILS:

Name: Age: Sex:

IP No. :

Smoker

Alcohol

District

State

Married

Occupation

Socio-economic status.

Education

ON ADMISSION:

Main Complaints :

Duration of Complaints :

Co – Morbid Illness :

Significant Past History :

CLINICAL EXAMINATION:

Pulse : BP :

RR : Temp :

Pallor : Icterus :

CVS :

RS :

P/A :

INVESTIGATIONS :

CBC : ESR :

Liver Function Test :

Renal Function Test :

CXR :

USG Abdomen

USG neck

HIV screening

Biopsy & HPE

AFB culture

GeneXpert

TREATMENT :

Intra – op Findings.

FOLLOW UP :

MASTER CHART

id_no	Sex	Age	ip_no	HIV_positive	previous_ATT	on_ATT	lymph_node
1	1	34	14131	1	1	0	1
2	0	25	16505	0	0	0	1
3	1	46	16430	0	0	0	1
4	1	33	16492	0	0	0	1
5	0	28	52943	0	0	0	1
6	0	19	52944	0	0	0	1
7	1	29	60102	0	0	0	1
8	0	50	59518	0	0	0	1
9	0	30	64058	0	0	0	1
10	0	19	64306	0	0	0	1
11	0	20	66023	0	0	0	1
12	0	25	67075	0	0	0	1
13	0	60	68684	0	0	0	1
14	0	27	69742	0	0	0	1
15	0	22	69814	0	1	0	1
16	0	45	67102	1	1	0	1
17	1	77	69933	0	0	0	1
18	0	22	69182	0	0	0	1
19	0	31	69723	0	0	0	1
20	1	29	229360	0	0	0	1
21	0	70	25366	0	0	0	1
22	0	26	64522	0	0	0	1
23	0	39	82891	0	1	0	1
24	1	21	81446	0	0	0	1
25	0	21	86915	0	0	0	1
26	0	19	87575	0	0	0	1
27	0	22	86375	0	1	0	1
28	0	38	87627	1	0	0	1
29	1	59	89577	0	0	0	1
30	1	37	90337	0	0	0	1
31	0	27	89455	0	0	0	1
32	0	19	85709	0	0	0	1
33	1	34	66734	0	0	1	1
34	0	28	89927	0	1	0	1
35	0	42	17129	0	0	0	1
36	1	37	89892	0	0	0	1
37	1	52	90929	0	0	0	1
38	0	19	10102	0	0	0	1
39	1	46	116413	0	0	0	1
40	1	33	11129	0	0	0	1
41	1	19	76729	0	0	1	1
42	0	19	92993	0	0	0	1
43	1	27	109742	0	0	0	1
44	0	22	10112	0	1	0	1
45	1	82	16993	0	0	0	1
46	1	32	122936	0	0	1	1
47	1	19	18691	0	0	0	1
48	0	38	187627	0	1	0	1
49	1	37	90338	0	0	0	1
50	0	22	186317	0	0	0	1

cervical_node	axillary_node	supra_clavicular_node	sub_mandibular_node	cold_abscess
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1	0	0	0	0
1	0	0	0	0
0	1	0	0	0
1	0	0	0	0
0	1	0	0	0
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HPE_TB	HPE_malinancy	HPE_reactive	Gene_expert_positive	AFB_c/s	tb_positive
0	0	1	0	0	0
0	0	1	0	0	0
1	0	0	1	1	1
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